Available online at www.sciencedirect.com
ScienceDirect
Neuromuscular Disorders 26 (2016) 551–559
www.elsevier.com/locate/nmd

Review

Opening the window: The case for carrier and perinatal screening for spinal muscular atrophy

Joseph K. Burns [a,b,c], Rashmi Kothary [a,c,d,e], Robin J. Parks [a,b,c,e,*]

[a] Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Canada
[b] Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Canada
[c] University of Ottawa Centre for Neuromuscular Disease, Ottawa, Canada
[d] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada
[e] Department of Medicine, University of Ottawa, Ottawa, Canada

Received 10 December 2015; received in revised form 20 June 2016; accepted 21 June 2016

Abstract

Spinal muscular atrophy (SMA) is the most common genetically inherited neurodegenerative disease that leads to infant mortality worldwide. SMA is caused by genetic deletion or mutation in the survival of motor neuron 1 (*SMN1*) gene, which results in a deficiency in SMN protein. For reasons that are still unclear, SMN protein deficiency predominantly affects α-motor neurons, resulting in their degeneration and subsequent paralysis of limb and trunk muscles, progressing to death in severe cases. Emerging evidence suggests that SMN protein deficiency also affects the heart, autonomic nervous system, skeletal muscle, liver, pancreas and perhaps many other organs. Currently, there is no cure for SMA. Patient treatment includes respiratory care, physiotherapy, and nutritional management, which can somewhat ameliorate disease symptoms and increase life span. Fortunately, several novel therapies have advanced to human clinical trials. However, data from studies in animal models of SMA indicate that the greatest therapeutic benefit is achieved through initiating treatment as early as possible, before widespread loss of motor neurons has occurred. In this review, we discuss the merit of carrier and perinatal patient screening for SMA considering the efficacy of emerging therapeutics and the physical, emotional and financial burden of the disease on affected families and society.

Keywords: Spinal muscular atrophy; Genetic screening; Therapy

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disease, which affects approximately 1 in 10,000 live births and has a carrier frequency of 1 in 40 people [1–3]. The hallmark of the disease is degenerative loss of α-motor neurons in the brain stem and spinal cord leading to progressive weakness of bulbar, trunk and limb muscles, and eventually paralysis in severe forms of the disease [3–5]. Thoracic cage deformation and respiratory muscle weakness can lead to respiratory failure, which frequently results in death, particularly in early onset cases which are typically more severe [6]. SMA causes the death of more infants than any other genetic disorder and is currently incurable [1,4]. Appropriately, this disease is the focus of numerous disease pathology and therapeutic research laboratories all over the world.

2. SMA genetics and disease severity

2.1. Genetic basis of SMA

SMA is caused by deletion or mutation of the survival of motor neuron 1 (*SMN1*) gene found on chromosome 5q in band 13 [7,8]. Homozygous deletion of *SMN1* occurs in approximately 95% of patients with SMA 5q13 [8], with the remainder displaying a heterozygous state in which one *SMN1* allele is deleted and the other contains a non-synonymous mutation, or more rarely two *SMN1* alleles that both contain intragenic mutations [9]. The functional loss of *SMN1* results in a deficiency of SMN protein; however the protein is not completely absent in affected patients due to the presence of *SMN2*, a second *SMN* gene also present on 5q13 [7]. The *SMN2* gene differs from *SMN1* by five nucleotides [7], but only one of these point mutations occurs in a coding region: the cytosine found at position +6 of exon 7 in *SMN1* is a thymidine in *SMN2*

* Corresponding author. Regenerative Medicine Program, Ottawa Hospital Research Institute, Room C4415, 501 Smyth Road, Ottawa, Ontario, Canada K1H 8L6. Fax: +1 613 737 8803.
E-mail address: rparks@ohri.ca (R.J. Parks).

http://dx.doi.org/10.1016/j.nmd.2016.06.459

[10]. Although this is a silent transition in terms of the amino acid encoded, the change to thymidine at this position results in a dramatic alteration in pre-mRNA splicing, such that exon 7 is excluded from ~90% of *SMN2* mature transcripts [11]. The protein generated from this alternatively spliced transcript, termed SMNΔ7, is unable to fulfill the functional roles of full-length SMN protein. A relatively small amount of full-length SMN protein is generated from the 10% of *SMN2* transcripts that include exon 7 [11]. No patients have been identified who have deletions or functional loss of both *SMN1* and *SMN2* [7], suggesting that loss of both genes results in embryonic lethality [12,13]. Mice encode only one *Smn* gene, and homozygous deletion of this gene is embryonic lethal [12]. In the absence of *SMN1*, the full-length SMN protein generated from *SMN2* is sufficient to prevent embryonic lethality but not sufficient to prevent the degeneration of α-motor neurons and the subsequent presentation of SMA. In general, disease severity is inversely correlated with the copy number of *SMN2* [14,15].

Multiple hypotheses exist regarding the mechanism by which the cytosine to thymidine change at position +6 of exon 7 causes its exclusion from the final transcript. Because this exon features weak splicing sites at both the 3′ and 5′ ends [16], proper inclusion of the exon is dependent on *cis* elements – DNA regions which are recognized by *trans*-acting splicing proteins and thereby enhance the inclusion or exclusion of a given element in a transcript. These elements include intronic splicing enhancers (ISEs), intronic splicing silencers (ISSs), exonic splicing enhancers (ESEs), and exonic splicing silencers (ESSs). At least one *cis* element from each category is believed to be involved in splicing of exon 7 in *SMN2* [17–25]. Thus, one therapeutic approach currently under development is targeting these various elements to enhance the probability of inclusion of exon 7 in pre-mRNA derived from *SMN2* (discussed below).

2.2. Variable severity and disease types

Patients with SMA are categorized into one of five types, depending on age of disease onset, peak motor function achieved, and life expectancy (Table 1) [28]. However, it is generally recognized that SMA presents as more of a continuum of phenotypes rather than discrete types [29]. Type 0 is characterized by congenital onset, respiratory distress at birth, and very low life expectancy. This type of SMA is generally immediately fatal if the patient does not receive respirator support at birth. Type 1, known as Werdnig–Hoffmann disease, presents before six months of age. Patients with type 1 SMA are unable to sit independently and life expectancy is less than two years [30]. In type 2, onset typically occurs between 6 and 18 months of age. These patients are able to sit independently but never achieve the ability to walk, and have life expectancies between 10 and 40 years. Patients with type 3, known as Kugelberg–Welander disease, experience disease onset after 18 months and ultimately achieve the ability to walk. Patients with type 4 SMA typically present as adults, exhibit wide phenotypic variability, and retain the ability to walk [31]. Types 3 and 4 are not usually associated with a reduced life expectancy [26]. For a historical perspective on the early identification of SMA, please see Reference [32].

Several groups have performed detailed observational studies on disease progression in patients with SMA types 1, 2 and 3 [33–37]. Natural history studies provide much needed information on the course of disease progression, which can aid in assigning appropriate clinical outcome measures in human trials of novel investigational approaches to SMA treatment or management. In general, the highest motor function achieved by a patient with SMA is more highly correlated to prognosis than age of onset [33]. Among patients with similar ages of onset, those who achieved the ability to sit or to walk independently lived longer than those who did not [33].

SMA type and prognosis are generally inversely correlated to the number of copies of the *SMN2* gene, an important modifier of disease severity [14,15,38]. The *SMN* locus is variably amplified and, in patients with increased *SMN2* copy number, an additive effect occurs in which the combined full-length transcript derived from each copy of the gene brings the patient closer to the levels of SMN present in an unaffected person. Quantitative PCR analysis performed by Feldkötter et al. determined the *SMN2* copy number of 375 patients with SMA of known type [27]. From these data, the authors generated percentage ratios that approximate the likelihood of each SMA subtype when the *SMN2* copy number is known (Table 1) [27]. For example, the likelihood of an affected child with only one copy of *SMN2* acquiring type 1 SMA is greater than 99.9%. With two copies of the gene, the probability of type 1 SMA is 97.26%, while the probabilities of type 2 and type 3 are 2.7% and 0.04%, respectively. With 3 or 4 copies of the *SMN2* gene, the patient is most likely to have type 2 and type 3 SMA, respectively (note: this study was not designed to detect more than 4 copies of *SMN2*). However, this study suggests that

Table 1
Clinical types of spinal muscular atrophy[a].

Type	Age of onset	Characteristic motor function	Life expectancy	Probability by copy number of *SMN2* (%)			
				1	2	3	4
0	Before birth	Reduced movement *in utero*	Fatal at birth	–[b]	–	–	–
1	<6 months	Unable to sit unsupported	<2 years	>99.9	97.3	7.2	1.6
2	6–18 months	Independent sitting	10–40 years	<0.01	2.7	82.8	14.8
3	>18 months	Independent walking	Normal	<0.01	0.04	10.0	83.6
4	>5 years	Normal walking	Normal	–	–	–	–

[a] Data from Russman [26] and Feldkötter [27], with modification.
[b] A dashed line indicates that data were not collected for this type.

SMN2 copy number is an imperfect predictor of phenotypic severity, particularly if the screen does not account for intragenic mutations within some copies of *SMN2* or incomplete duplications. For example, patients with 4 copies of *SMN2* can present with anywhere from type 1 to type 4 disease [27,39]. There are other genetic modifiers of SMA disease severity, such as *SERF1A* and *PLS3* [40–42], which further confounds a prediction of disease severity based solely on *SMN2* copy number (of note, the role of *PLS3* as a modifier of SMA disease severity has not been supported in some mouse models of SMA [43]). Thus, clinical assessment of peak motor function still provides one of the best predictors of disease severity and life expectancy.

3. SMN protein, putative functions and cell types affected

The SMN protein is 38 kDa in size, is ubiquitously expressed, and is found in both the cytoplasm and the nucleus [44,45]. Full-length SMN protein self-oligomerizes *via* association of a 30-amino acid domain encoded by exon 6, and missense mutations within or immediately adjacent to this domain inhibit the ability of the protein to self-associate [46]. The SMNΔ7 protein produced from the *SMN2* gene shows a greatly reduced ability to self-associate [46]. Following self-oligomerization, nuclear SMN is recruited into a macromolecular complex containing Gemin proteins. The formation of this complex appears to have a stabilizing effect on the SMN protein, and failure to self-oligomerize or to be recruited into these complexes may in part explain the reduced half-life of SMNΔ7 protein compared to the full-length protein [47]. The SMN complex is composed of SMN and Gemins 2 through 8 [48], and is found in subnuclear structures known as gems. The name is derived from Gemini, as the nuclear structures resembled twins of other previously identified coiled bodies in the nucleus [44]. The cellular localization of the complex appears to be influenced by its interaction with unr-interacting protein (Unrip), which directs localization to the cytoplasm and is absent in nuclear gems [49]. SMN appears to have many different functions, including roles in small nuclear ribonucleoprotein (snRNP) assembly and pre-mRNA splicing [50], translational regulation [51], R-loop resolution [52], transport of β-actin mRNA and secretion vesicles [53,54], and regulation of actin dynamics [55,56] (Table 2).

Although there is accumulating evidence that SMA is more than simply a disease of motor neurons, most studies in mouse models of SMA have focused on the effect of reduced levels of SMN on motor neuron function. These effects include altered splicing of many genes [50,72–74], poor terminal arborization [58], aggregation of neurofilaments in the motor end plate [58], impaired synaptic vesicle release [75], reduced neuromuscular junction plasticity [76] and, ultimately, denervation and death of the motor neuron [77]. All of these defects occur very early during disease progression, indicating that abnormal motor neuron structure and function are an early hallmark of the disease.

Although α-motor neurons are most affected in SMA, recent studies have shown that other tissues are also affected [78] (Table 2). Reduced levels of SMN in pre-symptomatic mice are associated with muscle weakness, and a delay in the appearance of mature isoforms of proteins important for muscle contractions [63]. Low levels of SMN cause muscle satellite cell defects, which manifests as the aberrant initiation of the differentiation program and reduced efficiency of myotube formation [62,63,79,80]. Similar observations were found in studies with myoblasts from patient with SMA [81]. Several studies have described cardiac muscle defects in mouse models of SMA [67–69], which are also observed in severe human disease [82]. Recently, defects in glucose metabolism and pancreatic function have been detected in SMA mice [64]. Indeed, heterozygous *Smn* +/− mice (mice naturally only have one copy of the *Smn* gene [12]) display defects in pancreas development and aberrant glucose metabolism, in the absence of any SMA-like disease, suggesting SMN may represent a diabetes susceptibility locus [65,83]. A recent study showed that reduced levels of SMN impact the ability of astrocytes to perform their normal functions of supporting neuron development and synapse formation [59]. Astrogliosis is

Table 2
Functions of SMN protein.

Function	SMA disease manifestation
Molecular function	
Small nuclear ribonucleoprotein (snRNP) assembly and pre-mRNA splicing	Reduced snRNP assembly and altered splicing pattern of many genes in brain and spinal cord [50]
Translational regulation	Altered protein translation (e.g. CARM1 [51])
R-loop resolution	Alterations in cellular gene expression and splicing [52]
Active transport	Altered transport of β-actin mRNA to motor neuron growth cones [53]; altered transport of Golgi-associated secretion vesicles [54]
Actin dynamics	Inappropriate activation of the RhoA/ROCK pathway perturbs actin dynamics [55,56]
Biological function	
Motor neuron outgrowth and axonal pathfinding	Impaired motor neuron connections in zebrafish [57]
Neuromuscular junction function and maturation	Abnormal endplate morphology, endplate denervation, neurofilament accumulation and disturbed function, impaired astrocyte and Schwann cell function [58–61]
Muscle development	Impaired satellite cell differentiation [62]; muscle weakness and delay in expression of mature proteins [63]
Pancreas development	Reduced β cell numbers leading to defective glucose metabolism [64,65]
Liver function	Altered expression of IGFALS leading to reduced stability of IGF-1 and stunted growth [66]
Autonomic nervous system	Cardiac defects and bradycardia [67–69]; vasodilation defects [70,71]

evident in end-stage SMA mice and in post-mortem patient spinal cords, and restoration of SMN specifically in astrocytes of SMA mice increased lifespan, improved motor unit function, and normalized neuromuscular junction defects. Intrinsic defects were also observed in Schwann cells deficient in SMN, which included reduced ability to myelinate neurons [60]. However, although restoration of SMN exclusively in Schwann cells did reverse the myelination defects in a mouse model of SMA, motor neuron survival was not improved [84]. Taken together, these studies demonstrate that although SMA may be considered primarily a disease of α-motor neurons, there are deficiencies that arise in other types of neurons, muscle, liver and pancreas that may contribute to the disease state. Since patients with SMA are now living longer due to improved assistive technology, these recently identified "secondary" deficiencies need to be more closely examined to evaluate their impact on the clinical course of SMA. Furthermore, truly effective therapies may need to restore SMN levels body-wide to achieve a complete beneficial effect.

4. Emerging therapies for SMA and the therapeutic window

Given the prevalence and potential severity of SMA, numerous research groups are working to identify novel therapies for this devastating disease (for review, please see References [78,85,86]). There are three broad areas to which therapies can be directed, including (1) correct the aberrant splicing of the *SMN2* gene product or stabilize the SMNΔ7 protein; (2) target the downstream damage caused by SMN deficiency by protecting affected tissues from death; or (3) reintroduce the missing *SMN1* gene or its gene product using a therapeutic vector. Within each of these strategies, there exists a wide variety of therapeutics in development (Table 3), some of which have entered clinical trials. For example, an antisense oligonucleotide therapeutic developed by IONIS Pharmaceuticals (formerly ISIS Pharmaceuticals) and Biogen Idec designed to block splicing-out of exon 7 from the *SMN2* transcript, has completed a phase I clinical trial in 28 patients [87,88]. The agent was well tolerated and appeared to improve motor function in some of the treated patients. In the follow-up open-label Phase II study of children with SMA, IONIS observed a time- and dose-dependent increase in muscle function scores in children treated with multiple-doses of their lead oligonucleotide ISIS-SMN_{Rx} or nusinersen [89]. No severe adverse events were reported. Nusinersen efficacy is currently being assessed in a Phase III clinical trial [90]. A self-complementary adeno-associated viral vector based on serotype 9 (sc-AAV9) encoding a full-length copy of the *SMN1* cDNA, which showed very promising results in mouse models of SMA [91], is currently in Phase I/II clinical trial in children with SMA Type I [92]. Preliminary results from this study showed that the vector, AVXS-101, appears to be generally safe and well tolerated in the nine patients studied to date, and all patients experienced an improvement in motor function [93]. Thus, at least two therapies are showing promise in clinical trials for SMA.

A central theme that has emerged from preclinical studies of novel therapies in SMA mice is the importance of early intervention to achieve maximal therapeutic benefit. Antisense oligonucleotides that rescue the phenotype in SMA model mice must be administered *in utero* or immediately upon birth to achieve the greatest beneficial effect [66,94,95]. Similarly, studies utilizing the scAAV9-*SMN1* showed that the vector had to be delivered very soon after birth of the affected mice in order to achieve a therapeutic benefit and extend mouse survival [91]. The therapeutic window for severe SMA in human patients is believed to be early and narrow, to prevent the rapid degeneration of the α-motor neurons and other affected tissues. Therefore, early detection is essential to identify candidates for the many therapeutics currently approaching or entering the clinic [3,96,97].

Table 3
Therapeutic strategies for treating SMA.

Category	Sub-class	Mode of action	Agent
SMN2 targeting	Histone deacetylase inhibition	Increases transcription and/or splicing	Valproic acid, sodium butyrate, trichostatin A, SAHA, LBH589
	Counteract DNA methylation	Reduces silencing of SMN2	Romidepsin, vorinostat
	Promoter activation	Increase transcription of SMN2	Prolactin, quinazalone 495
	Antisense oligonucleotide	Alter SMN2 pre-mRNA splicing	Pro-105, ASO-10-27
	Bifunctional RNA	Recruit splicing factors to optimize splicing of SMN2	Various
	Trans-splicing	Provide a splice acceptor and SMN1 exon 7	Various
	Drugs affecting SMN2 pre-mRNA splicing	Promote SMN2 pre-mRNA splicing to include exon 7	Hydroxyurea, PTK-SMA1, albuterol/salbutamol, aclarubicin
	Reduce protein turnover	Enhance protein stability or reduce degradation	Bortezomib
	Aminoglycosides	Promotes read-through of SMN2 exon 8, creating a more stable protein	G418, TC007, tobramycin, amikacin
Non-SMN targeting	Regulation of actin dynamics	Rho kinase inhibition	Y-27632, fasudil
	Regulation of actin dynamics	Enhance axonogenesis through increasing F-actin levels	Plastin 3
	Enhance muscle strength	Myostatin inhibition	Follistatin, tirasemtiv
	Protect motor neurons	Neurotrophic factor	Cardiotrophin-1, β-lactam, IGF-1
Replacement of SMN1	Gene replacement	Replace endogenous gene	Vector-mediated delivery of a cDNA copy of *SMN1*
	Stem cell therapies	Differentiate into motor neurons to replace lost cells or provide neuroprotective functions	Neural stem cells, embryonic stem cell-derived neural stem cells

5. Genetic screening for SMA: cost versus benefit

5.1. Genetic testing and carrier screening

Considering the brevity of the therapeutic window for patients with the most severe form of SMA, early detection of SMA will likely be essential to the success of any therapeutic regimen. Although the merit of universal prenatal/neonatal screening for SMA is currently being debated, carrier screening is presently performed for parents of an affected child in an attempt to predict the risk of recurrence in future children [98]. In addition to confirming their carrier status, parents of an affected child are assessed for their *SMN2* copy number in an effort to predict the severity of SMA and prognosis in a potentially affected child, although, as discussed above, there is an imperfect correlation between *SMN2* copy number and disease prognosis.

There are a number of methods available to screen DNA for *SMN1* deletion or *SMN2* copy number (for an overview, please see Reference [97]). Perhaps the most suitable screening method among current technologies for hospital-based testing is PCR/restriction enzyme digestion analysis. This method allows for unambiguous detection of *SMN1* deletion [99], however, the method does not detect point mutations resulting in functional loss of *SMN1*, and therefore additional diagnostic tests are necessary when the carriers exhibit such mutations. An emerging technology, which identifies *SMN1* point mutations and gene dosage of *SMN2*, is high resolution melting analysis (HRMA) [100]. HRMA analysis is relatively quick, and amenable to high throughput capabilities, which makes this assay perhaps best suited for population-based screening. New techniques such as whole exome or whole genome sequencing are fundamentally changing the way novel disease-causing genes are identified and patients diagnosed [101], and it is likely that in the future these technologies will impact diagnosis of SMA. However, currently these techniques are ineffective at distinguishing *SMN1* from *SMN2* or *SMN2* copy number.

5.2. The debate over population-based screening for SMA

There is debate regarding the utility of genetic screening for SMA primarily due to the limitations of carrier screening and the incurable status of the disease. Opponents of population-based SMA screening cite the significant costs of widespread screening [102], the potential for false negatives in carrier testing [96], and the concern that carrier status testing is only relevant for couples preparing to conceive a child together. SMA carrier status does not affect the health of the individual, and knowing that one is a carrier might affect the decision to have children. The prospect of widespread screening is also given pause by the absence of a cure for the disease. Screening for SMN violates the second of ten criteria adopted by the World Health Organization (WHO) in 1968 (the Wilson–Jungner criteria), which states that in order for a genetic screen to be warranted, "there should be an accepted treatment for patients with recognized disease" [103]. The American Congress of Obstetricians and Gynecologists (ACOG) cautions against such screening particularly in the prenatal case, as improper understanding of the variability in disease phenotype may lead to poorly informed terminations of pregnancy [104].

Several groups have argued in favor of population-based carrier screening. Due to the high carrier frequency and clinical severity of the disease, the American College of Medical Genetics (ACMG) recommends population-based SMA carrier screening in addition to screening of all members of an affected family [2]. The Muscular Dystrophy Association (MDA) has advocated that genetic diseases for which new therapeutics are entering clinical study, such as SMA, ought to be granted priority in the review process used to determine which diseases merit widespread newborn screening [105]. While it is true that no accepted treatment for SMA currently exists, early detection of SMA by a prenatal/neonatal screen could improve quality of life for the patient by allowing the parents to anticipate immediate health needs and prepare for the proper medical care of the affected child [106].

A recent reassessment of the Wilson–Jungner criteria published by the WHO recognized the benefits of genetic screening for disease which lack an accepted treatment, and a new set of tentative criteria was generated based on this consideration and on other new developments in genomics [107]. This new set of criteria does not require a specific established treatment for a disease, but instead requires an established need for the screen, a clear target population, and the integration of patient education in the screening program, none of which is an obstacle to SMA screening [107]. Considering these more modern ethical criteria and the current state of screening technology, widespread screening for SMA certainly warrants further discussion.

5.3. Financial cost of SMA

Cost of illness (also referred to as burden of disease) refers to the financial burden incurred by the patient, the patient's family, and insurance companies to fund treatment of the disease and non-medical accommodations related to the illness. A recent study by the Lewin group, funded by the MDA, evaluated the annual cost of illness of several muscular disorders including hereditary progressive muscular dystrophy (HPMD), myotonic dystrophy (MMD), congenital muscular dystrophy (CMD), amyotrophic lateral sclerosis (ALS), and SMA [108,109]. Patients with SMA were not separated by disease type, but instead were divided into patients whose disease onset occurred prior to three years of age (SMA Early Onset) or later (SMA Other). The mean medical expenditure for privately insured patients in the SMA Early Onset category was $121,682 USD annually (all numbers are given in US dollars), substantially higher than the means for the other diseases (CMD was second highest with $32,341). However, the sample size for the SMA Early Onset category was small and there was considerable variability between patients (standard error = $98,898, n = 14). Since many patients who experience early onset of SMA exhibit type 1 disease, the severity of the disease and the acute medical attention required for these patients may account for such high medical expenses. Mean annual medical costs for patients in the SMA Other category

were lower ($20,085, standard error = $1,811), but similar to the other muscular diseases [108,109].

In addition to funds spent on direct medical diagnosis and treatment, patients with SMA and their families may incur non-medical expenses, such as wage loss, caregiver salaries and cost of assistive devices to enhance the patient's mobility and independence. An important cost to consider is wages lost by the patient or the patient's family, particularly for the SMA Early Onset group, in which 81.45% of patients required 16 or more hours of attendance per day [108,109]. The estimated annual loss of income for parents and guardians of patients with early-onset SMA, with a weighted average which considers wages lost and the time cost for sustained attendance of the patient, is $35,623 (standard error = $2462), considerably higher than the second highest wage loss ($19,217 for ALS). The higher level of mobility associated with later onset of SMA is expected to cause the lower wage loss in the SMA Other group, with a mean loss of $11,110 (standard error = $627). The authors reported the mean annual cost for other non-medical expenditures, including home modifications, vehicle modifications, and non-medical professional care. Annual costs for each patient were obtained by dividing the total non-medical costs over the patient's life by the number of years the patient was affected. Again, the mean cost was highest for the SMA Early Onset group at $51,665, but these costs varied significantly between patients and the sample size was again small (standard error = $39,501, n = 17). The majority of these funds were used to pay professional caregivers. Patients in the SMA Other group averaged $14,295 for non-medical expenses (standard error = $1651), which was similar to the costs incurred by the other muscular diseases [108,109].

Finally, the Lewin group estimated total national cost of illness in the United States for each disease by multiplying the per capita medical expense by three estimations of disease prevalence in the country. Using the moderate estimations for disease prevalence, this yields a total medical cost of $684 million annually for early-onset SMA and $273 million for SMA with an onset of later than three years [108,109]. Since SMA occurs at a similar rate across ethnicities [110], these costs can be expected to be proportionately similar in other countries, although costs of medical services will vary by country. Obviously, the significant medical and supportive costs incurred in caring for patients with SMA underscore the need for effective therapeutics and early intervention.

5.4. Cost effectiveness of SMA screening

In order to assess the cost effectiveness of prenatal SMA screening, Little et al. developed a theoretical model comparing universal screening to complete absence of screening using values derived from literature [102]. This study took into account expenses such as carrier testing, amniocentesis, fetal testing, and pregnancy termination in instances of a positive fetal test, versus the total lifetime cost of caring for a child with severe or mild disease. The authors also took into account maternal quality-adjusted life years (QALYs), a measure of disease burden on quality of life lived. Little et al. estimated the total cost of universal screening to be $44 million per 100,000 women, or approximately $5 million per SMA case averted. In contrast, the total cost for treating the 10 affected children (per 100,000 women) predicted to be born in the absence of screening would be about $4.7 million. The authors ultimately concluded that universal prenatal screening was not cost effective; however, their study suggested that for populations at high risk, such as those with a family history of SMA, carrier testing may still be a cost-effective strategy.

6. Final perspective

Spinal muscular atrophy can cause tremendous suffering – physical, financial, and emotional – to the patient and the family of the affected individual. Currently, from an economic perspective, universal testing for SMA does not appear a viable choice. However, testing of individuals at high risk, such as those with a family history of SMA, is prudent. A positive result from carrier screening in parents can lead to prenatal screening of the child. An unborn child confirmed to have homozygous deletion or mutation in *SMN1* can be tested for *SMN2* gene dosage. Although *SMN2* gene dosage is not a perfect predictor of SMA disease type, given proper counseling and support, the family of an affected child could consider the probability of their child being born with severe disease and tailor their response accordingly. This information would assist the parents in making an informed decision in the unfortunate event that the prognosis for their unborn child is extremely poor, or make appropriate preparations, both practically and emotionally, for a child whose life expectancy is normal, but will require additional attendance and assistance with mobility.

SMA currently has no effective treatment or cure, but several experimental therapeutics have reached advanced stage of clinical testing in human patients. All available data from studies in animal models of SMA have suggested that maximum benefit is achieved only when the therapy is initiated prior to overt loss of motor neurons. It is logical to assume that the same will occur in human patients, indicating that early detection of SMA is desirable either to initiate therapy very soon after birth, or perhaps even *in utero* in the case of a child with severe SMA. Prenatal screening will identify affected children before the closure of the therapeutic window, allowing these patients to be treated with emerging SMA therapeutics.

Any argument for or against prenatal or neonatal screening for SMA that is based solely on economics has not taken into account the very legitimate issues of parent preparedness and potential for early therapeutic intervention. As technology progresses, and the cost of screening for SMA becomes less and new therapeutics become available, it will be important to readdress the need for SMA screening. Although SMA is presently incurable, prenatal or neonatal screening will be a boon to quality of life for patients and a crucial catalyst for the development of treatments and possibly a cure.

Acknowledgements

Research in the Parks laboratory is supported by grants from the Canadian Institutes of Health Research (CIHR) (MOP-130279 to R.K.; ERL-138414 to R.K.; MOP-136898 to

R.J.P.; MOP-142316 to R.J.P.), the Natural Sciences and Engineering Research Council of Canada (NSERC) (RGPIN-2014-04810 to R.J.P.), and the Cancer Research Society (Canada) (19363 to R.J.P.). Research in the Kothary laboratory is supported by grants from CIHR, the Muscular Dystrophy Association (USA) (294568 to R.K.), Cure SMA/Families of SMA Canada (KOT1617 to R.K.), and the Multiple Sclerosis Society of Canada (EGID2098 to R.K.). J.K.B. was supported by the Queen Elizabeth II Graduate Scholarship in Science and Technology from the Government of Ontario. R.K. is a recipient of a University Health Research Chair from the University of Ottawa. We thank Drs. Jane Larkindale, Dennis Bulman (Children's Hospital of Eastern Ontario Research Institute) and Jodi Warman Chardon (The Ottawa Hospital) for providing data and insight for this review.

References

[1] Crawford TO, Pardo CA. The neurobiology of childhood spinal muscular atrophy. Neurobiol Dis 1996;3:97–110.
[2] Prior TW. Carrier screening for spinal muscular atrophy. Genet Med 2008;10:840–2.
[3] Nurputra DK, Lai PS, Harahap NI, et al. Spinal muscular atrophy: from gene discovery to clinical trials. Ann Hum Genet 2013;77:435–63.
[4] Shababi M, Lorson CL, Rudnik-Schoneborn SS. Spinal muscular atrophy: a motor neuron disorder or a multi-organ disease? J Anat 2014;224:15–28.
[5] Boyer JG, Bowerman M, Kothary R. The many faces of SMN: deciphering the function critical to spinal muscular atrophy pathogenesis. Future Neurol 2010;5:873–90.
[6] Rudnik-Schoneborn S, Stolz P, Varon R, et al. Long-term observations of patients with infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). Neuropediatrics 2004;35:174–82.
[7] Lefebvre S, Bürglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 1995;80:155–65.
[8] Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet 1995;4:1927–33.
[9] Rochette CF, Surh LC, Ray PN, et al. Molecular diagnosis of non-deletion SMA patients using quantitative PCR of SMN exon 7. Neurogenetics 1997;1:141–7.
[10] Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc Natl Acad Sci USA 1999;96:6307–11.
[11] Jodelka FM, Ebert AD, Duelli DM, Hastings ML. A feedback loop regulates splicing of the spinal muscular atrophy-modifying gene, SMN2. Hum Mol Genet 2010;19:4906–17.
[12] Schrank B, Gotz R, Gunnersen JM, et al. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci USA 1997;94:9920–5.
[13] Hsieh-Li H, Chang JG, Jong YJ, et al. A mouse model for spinal muscular atrophy. Nat Genet 2000;24:66–70.
[14] Lefebvre S, Burlet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 1997;16:265–9.
[15] Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 1997;6:1205–14.
[16] Lim SR, Hertel KJ. Modulation of survival motor neuron pre-mRNA splicing by inhibition of alternative 3′ splice site pairing. J Biol Chem 2001;276:45476–83.
[17] Hofmann Y, Lorson CL, Stamm S, Androphy EJ, Wirth B. Htra2-Beta1 stimulates an exonic splicing enhancer and can restore full-length SMN expression to survival motor neuron 2 (SMN2). Proc Natl Acad Sci USA 2000;97:9618–23.
[18] Hofmann Y, Wirth B. hnRNP-G promotes exon 7 inclusion of survival motor neuron (SMN) via direct interaction with Htra2-Beta1. Hum Mol Genet 2002;11:2037–49.
[19] Cartegni L, Krainer AR. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN. Nat Genet 2002;30:377–84.
[20] Kashima T, Manley JL. A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat Genet 2003;34:460–3.
[21] Pedrotti S, Bielli P, Paronetto MP, et al. The splicing regulator Sam68 binds to a novel exonic splicing silencer and functions in SMN2 alternative splicing in spinal muscular atrophy. EMBO J 2010;29:1235–47.
[22] Singh NN, Singh RN, Androphy EJ. Modulating role of RNA structure in alternative splicing of a critical exon in the spinal muscular atrophy genes. Nucleic Acids Res 2007;35:371–89.
[23] Miyajima H, Miyaso H, Okumura M, Kurisu J, Imaizumi K. Identification of a cis-acting element for the regulation of SMN exon 7 splicing. J Biol Chem 2002;277:23271–7.
[24] Miyaso H, Okumura M, Kondo S, Higashide S, Miyajima H, Imaizumi K. An intronic splicing enhancer element in survival motor neuron (SMN) pre-mRNA. J Biol Chem 2003;278:15825–31.
[25] Kashima T, Rao N, Manley JL. An intronic element contributes to splicing repression in spinal muscular atrophy. Proc Natl Acad Sci USA 2007;104:3426–31.
[26] Russman BS. Spinal muscular atrophy: clinical classification and disease heterogeneity. J Child Neurol 2007;22:946–51.
[27] Feldkötter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am J Hum Genet 2002;70:358–68.
[28] Dubowitz V. Chaos in classification of the spinal muscular atrophies of childhood. Neuromuscul Disord 1991;1:77–80.
[29] Dubowitz V. Chaos in the classification of SMA: a possible resolution. Neuromuscul Disord 1995;5:3–5.
[30] Thomas NH, Dubowitz V. The natural history of type I (severe) spinal muscular atrophy. Neuromuscul Disord 1994;4:497–502.
[31] Lunn MR, Wang CH. Spinal muscular atrophy. Lancet 2008;371:2120–33.
[32] Dubowitz V. Ramblings in the history of spinal muscular atrophy. Neuromuscul Disord 2009;19:69–73.
[33] Russman BS, Iannacone ST, Buncher CR, et al. Spinal muscular atrophy: new thoughts on the pathogenesis and classification schema. J Child Neurol 1992;7:347–53.
[34] Russman BS, Melchreit R, Drennan JC. Spinal muscular atrophy: the natural course of disease. Muscle Nerve 1983;6:179–81.
[35] Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology 2014;83:810–17.
[36] Kaufmann P, McDermott MP, Darras BT, et al. Prospective cohort study of spinal muscular atrophy types 2 and 3. Neurology 2012;79:1889–97.
[37] Kaufmann P, McDermott MP, Darras BT, et al. Observational study of spinal muscular atrophy type 2 and 3: functional outcomes over 1 year. Arch Neurol 2011;68:779–86.
[38] Moulard B, Salachas F, Chassande B, et al. Association between centromeric deletions of the SMN gene and sporadic adult-onset lower motor neuron disease. Ann Neurol 1998;43:640–4.
[39] Crawford TO, Paushkin SV, Kobayashi DT, et al. Evaluation of SMN protein, transcript, and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. PLoS ONE 2012;7:e33572.
[40] Brkusanin M, Kosac A, Jovanovic V, et al. Joint effect of the SMN2 and SERF1A genes on childhood-onset types of spinal muscular atrophy in Serbian patients. J Hum Genet 2015;60(11):723–8.
[41] Stratigopoulos G, Lanzano P, Deng L, et al. Association of plastin 3 expression with disease severity in spinal muscular atrophy only in postpubertal females. Arch Neurol 2010;67:1252–6.

[42] Oprea GE, Krober S, McWhorter ML, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science 2008;320:524–7.
[43] McGovern VL, Massoni-Laporte A, Wang X, et al. Plastin 3 expression does not modify spinal muscular atrophy severity in the 7 SMA mouse. PLoS ONE 2015;10:e0132364.
[44] Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. EMBO J 1996;15:3555–65.
[45] Fischer U, Liu Q, Dreyfuss G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 1997;90:1023–9.
[46] Lorson CL, Strasswimmer J, Yao JM, et al. SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat Genet 1998;19: 63–6.
[47] Burnett BG, Munoz E, Tandon A, Kwon DY, Sumner CJ, Fischbeck KH. Regulation of SMN protein stability. Mol Cell Biol 2009;29:1107–15.
[48] Kolb SJ, Battle DJ, Dreyfuss G. Molecular functions of the SMN complex. J Child Neurol 2007;22:990–4.
[49] Grimmler M, Otter S, Peter C, Muller F, Chari A, Fischer U. Unrip, a factor implicated in cap-independent translation, associates with the cytosolic SMN complex and influences its intracellular localization. Hum Mol Genet 2005;14:3099–111.
[50] Zhang Z, Lotti F, Dittmar K, et al. SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. Cell 2008;133:585–600.
[51] Sanchez G, Dury AY, Murray LM, et al. A novel function for the survival motoneuron protein as a translational regulator. Hum Mol Genet 2013;22:668–84.
[52] Zhao DY, Gish G, Braunschweig U, et al. SMN and symmetric arginine dimethylation of RNA polymerase II C-terminal domain control termination. Nature 2016;529:48–53.
[53] Rossoll W, Jablonka S, Andreassi C, et al. SMN, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol 2003;163:801–12.
[54] Ting CH, Wen HL, Liu HC, Hsieh-Li HM, Li H, Lin-Chao S. The spinal muscular atrophy disease protein SMN is linked to the Golgi network. PLoS ONE 2012;7:e51826.
[55] Bowerman M, Anderson CL, Beauvais A, Boyl PP, Witke W, Kothary R. SMN, profilin IIa and plastin 3: a link between the deregulation of actin dynamics and SMA pathogenesis. Mol Cell Neurosci 2009;42:66–74.
[56] Bowerman M, Beauvais A, Anderson CL, Kothary R. Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. Hum Mol Genet 2010;19:1468–78.
[57] Zhang HL, Pan F, Hong D, Shenoy SM, Singer RH, Bassell GJ. Active transport of the survival motor neuron protein and the role of exon-7 in cytoplasmic localization. J Neurosci 2003;23:6627–37.
[58] Kariya S, Park GH, Maeno-Hikichi Y, et al. Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. Hum Mol Genet 2008;17:2552–69.
[59] Rindt H, Feng Z, Mazzasette C, et al. Astrocytes influence the severity of spinal muscular atrophy. Hum Mol Genet 2015;24:4094–102.
[60] Hunter G, Aghamaleky Sarvestany A, Roche SL, Symes RC, Gillingwater TH. SMN-dependent intrinsic defects in Schwann cells in mouse models of spinal muscular atrophy. Hum Mol Genet 2014;23: 2235–50.
[61] Murray LM, Comley LH, Thomson D, Parkinson N, Talbot K, Gillingwater TH. Selective vulnerability of motor neurons and dissociation of pre- and post-synaptic pathology at the neuromuscular junction in mouse models of spinal muscular atrophy. Hum Mol Genet 2008;17:949–62.
[62] Hayhurst M, Wagner AK, Cerletti M, Wagers AJ, Rubin LL. A cell-autonomous defect in skeletal muscle satellite cells expressing low levels of survival of motor neuron protein. Dev Biol 2012;368: 323–34.
[63] Boyer JG, Murray LM, Scott K, De Repentigny Y, Renaud JM, Kothary R. Early onset muscle weakness and disruption of muscle proteins in mouse models of spinal muscular atrophy. Skelet Muscle 2013;3:24.
[64] Bowerman M, Swoboda KJ, Michalski JP, et al. Glucose metabolism and pancreatic defects in spinal muscular atrophy. Ann Neurol 2012;72: 256–68.
[65] Bowerman M, Michalski JP, Beauvais A, Murray LM, DeRepentigny Y, Kothary R. Defects in pancreatic development and glucose metabolism in SMN-depleted mice independent of canonical spinal muscular atrophy neuromuscular pathology. Hum Mol Genet 2014;23:3432–44.
[66] Hua Y, Sahashi K, Rigo F, et al. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature 2011;478:123–6.
[67] Bevan AK, Hutchinson KR, Foust KD, et al. Early heart failure in the SMNDelta7 model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery. Hum Mol Genet 2010;19:3895–905.
[68] Shababi M, Habibi J, Ma L, Glascock JJ, Sowers JR, Lorson CL. Partial restoration of cardio-vascular defects in a rescued severe model of spinal muscular atrophy. J Mol Cell Cardiol 2012;52:1074–82.
[69] Gogliotti RG, Quinlan KA, Barlow CB, Heier CR, Heckman CJ, Didonato CJ. Motor neuron rescue in spinal muscular atrophy mice demonstrates that sensory-motor defects are a consequence, not a cause, of motor neuron dysfunction. J Neurosci 2012;32:3818–29.
[70] Hachiya Y, Arai H, Hayashi M, et al. Autonomic dysfunction in cases of spinal muscular atrophy type 1 with long survival. Brain Dev 2005;27:574–8.
[71] Araujo A, Araujo M, Swoboda KJ. Vascular perfusion abnormalities in infants with spinal muscular atrophy. J Pediatr 2009;155:292–4.
[72] Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G. A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell 1998;95:615–24.
[73] Liu H, Shafey D, Moores JN, Kothary R. Neurodevelopmental consequences of Smn depletion in a mouse model of spinal muscular atrophy. J Neurosci Res 2010;88:111–22.
[74] Lotti F, Imlach WL, Saieva L, et al. An SMN-dependent U12 splicing event essential for motor circuit function. Cell 2012;151:440–54.
[75] Kong L, Wang X, Choe DW, et al. Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. J Neurosci 2009;29:842–51.
[76] Murray LM, Beauvais A, Bhanot K, Kothary R. Defects in neuromuscular junction remodelling in the Smn2B/- mouse model of spinal muscular atrophy. Neurobiol Dis 2013;49:57–67.
[77] McGovern VL, Gavrilina TO, Beattie CE, Burghes AH. Embryonic motor axon development in the severe SMA mouse. Hum Mol Genet 2008;17:2900–9.
[78] Goulet BB, Kothary R, Parks RJ. At the “junction” of spinal muscular atrophy pathogenesis: the role of neuromuscular junction dysfunction in SMA disease progression. Curr Mol Med 2013;13:1160–74.
[79] Bricceno KV, Martinez T, Leikina E, et al. Survival motor neuron protein deficiency impairs myotube formation by altering myogenic gene expression and focal adhesion dynamics. Hum Mol Genet 2014;23: 4745–57.
[80] Boyer JG, Deguise MO, Murray LM, et al. Myogenic program dysregulation is contributory to disease pathogenesis in spinal muscular atrophy. Hum Mol Genet 2014;23:4249–59.
[81] Martinez-Hernandez R, Soler-Botija C, Also E, et al. The developmental pattern of myotubes in spinal muscular atrophy indicates prenatal delay of muscle maturation. J Neuropathol Exp Neurol 2009;68: 474–81.
[82] Rudnik-Schoneborn S, Heller R, Berg C, et al. Congenital heart disease is a feature of severe infantile spinal muscular atrophy. J Med Genet 2008;45:635–8.
[83] Davis RH, Miller EA, Zhang RZ, Swoboda KJ. Responses to fasting and glucose loading in a cohort of well children with spinal muscular atrophy type II. J Pediatr 2015;167:1362–8, e1.
[84] Hunter G, Powis RA, Jones RA, et al. Restoration of SMN in Schwann cells reverses myelination defects and improves neuromuscular function in spinal muscular atrophy. Hum Mol Genet 2016;doi:10.1093/hmg/ddw141.
[85] d’Ydewalle C, Sumner CJ. Spinal muscular atrophy therapeutics: where do we stand? Neurother 2015;12:303–16.

[86] Faravelli I, Nizzardo M, Comi GP, Corti S. Spinal muscular atrophy–recent therapeutic advances for an old challenge. Nat Rev Neurol 2015;11:351–9.
[87] Chiriboga CA, Swoboda KJ, Darras BT, et al. Results from a phase 1 study of nusinersen (ISIS-SMNRx) in children with spinal muscular atrophy. Neurology 2016;86:890–7.
[88] Hache M, Swoboda KJ, Sethna N, et al. Intrathecal injections in children with spinal muscular atrophy: nusinersen clinical trial experience. J Child Neurol 2016;31:899–906.
[89] IONIS-Pharmaceuticals. Isis pharmaceuticals reports data from ISIS-SMN Rx phase 2 studies in infants and children with spinal muscular atrophy, <http://ir.isispharm.com/phoenix.zhtml?c=222170&p=irol-newsArticle&ID=1976144>; 2014 [accessed 27.05.15].
[90] ClinicalTrials.gov. A study to assess the efficacy and safety of ISIS-SMN Rx in infants with spinal muscular atrophy, <https://clinicaltrials.gov/ct2/show/NCT02193074?term=spinal+muscular+atrophy&rank=13>; 2015 [accessed 27.05.15].
[91] Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol 2010;28:271–4.
[92] ClinicalTrials.gov. Gene transfer clinical trial for spinal muscular atrophy type 1, <https://clinicaltrials.gov/ct2/show/NCT02122952?term=NCT02122952&rank=1>; 2015 [accessed 27.05.15].
[93] Avexis. Data from ongoing study of AVXS-101 in spinal muscular atrophy type 1, Presented at World Muscle Congress, <http://investors.avexis.com/phoenix.zhtml?c=254285&p=irol-newsArticle&ID=2127680>; 2015 [accessed 27.06.16].
[94] Hua Y, Sahashi K, Hung G, et al. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. Genes Dev 2010;24:1634–44.
[95] Porensky PN, Mitrpant C, McGovern VL, et al. A single administration of morpholino antisense oligomer rescues spinal muscular atrophy in mouse. Hum Mol Genet 2012;21:1625–38.
[96] Prior TW, Snyder PJ, Rink BD, et al. Newborn and carrier screening for spinal muscular atrophy. Obstet Gynecol Surv 2010;65:697–9.
[97] Phan HC, Taylor JL, Hannon H, Howell R. Newborn screening for spinal muscular atrophy: anticipating an imminent need. Semin Perinatol 2015;39:217–29.
[98] Matthijs G, Devriendt K, Fryns JP. The prenatal diagnosis of spinal muscular atrophy. Prenat Diagn 1998;18:607–10.
[99] van der Steege G, Grootscholten PM, van der Vlies P, et al. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy 7. Lancet 1995;345:985–6.
[100] Chen WJ, Dong WJ, Lin XZ, et al. Rapid diagnosis of spinal muscular atrophy using high-resolution melting analysis. BMC Med Genet 2009;10:45.
[101] Lek M, MacArthur D. The challenge of next generation sequencing in the context of neuromuscular diseases. J Neuromuscul Dis 2014;1:135–49.
[102] Little SE, Janakiraman V, Kaimal A, Musci T, Ecker J, Caughey AB. The cost-effectiveness of prenatal screening for spinal muscular atrophy. Am J Obstet Gynecol 2010;202:253, e1-7.
[103] Orzalesi M, Danhaive O. Ethical problems with neonatal screening. Ann Ist Super Sanita 2009;45:325–30.
[104] ACOG. ACOG committee opinion no. 432: spinal muscular atrophy. Obstet Gynecol 2009;113:1194–6.
[105] MDA. MDA's board chairman advocates support for newborn screening, [news article], 2013 March 7, <http://quest.mda.org/news/mda-board-chairman-advocates-support-newborn-screening>; 2014 [accessed 27.06.16].
[106] Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: relation to age, SMN2 copy number, and function. Ann Neurol 2005;57:704–12.
[107] Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ 2008;86:317–19.
[108] MDA. Cost of amyotrophic lateral sclerosis, muscular dystrophy, and spinal muscular atrophy in the United States. In: Muscular dystrophy association sponsored study prepared by The Lewin Group. Tucson, AZ: MDA; 2012. p. 1–54.
[109] Larkindale J, Yang W, Hogan PF, et al. Cost of illness for neuromuscular diseases in the United States. Muscle Nerve 2014;49:431–8.
[110] Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72 400 specimens. Eur J Hum Genet 2012;20:27–32.